AD_____

Award Number: W81XWH-11-1-0561

TITLE:

Use of the Photo-Electromyogram to Objectively Diagnose and Monitor Treatment of Post-TBI Light Sensitivity

PRINCIPAL INVESTIGATOR: Randy Kardon M.D. Ph.D.

CONTRACTING ORGANIZATION: University of Iowa
 Iowa City, IA 52242-1316

REPORT DATE: October 2014

TYPE OF REPORT:ÁAnnual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
               Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

    x Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2014	Annual	Sep. 6, 2013 - Sep. 5, 2014

4. TITLE AND SUBTITLE
Use of the Photo-Electromyogram to Objectively Diagnose and Monitor Treatment of Post-TBI Light Sensitivity

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0561

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Randy Kardon MD PhD, Andrew Russo PhD, Pieter Poolman PhD

E-Mail: randy-kardon@uiowa.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME (S) AND ADDRESS(ES)
University of Iowa
Iowa City, IA 52242

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Purpose: to test the whether photosensitivity (photophobia) after traumatic brain injury (TBI) is due to increased sensitivity of the brainstem trigeminal sensory nucleus, as revealed objectively by an exaggerated photoblink reflex (photo-electromyogram). This will be tested in humans and in a mouse strain genetically engineered to be hypersensitive to calcitonin gene related peptide (CGRP), the neurotransmitter modulating trigeminal nerve function.
Scope: objective methods to quantify photo-sensitivity include 1) light evoked potentials (electromyogram) from the blinking and squinting muscles of the forehead 2) the pupil light reflex 3) light evoked changes in sympathetic nerve activity, measured by changes in skin conductance and heart rate.
Major Findings (Year 3): 1) based on Facial Action Coding System (FACS) metrics extracted from video recordings, we have shown that videography and image analysis -- for tracking changes in eyelid position and eye brow shape -- can be used as a substitute method for "measuring" EMG activity unobtrusively, 2) in contrast to earlier results, we have observed an overall decrease in EMG activity after CGRP administration, especially for hRAMP1 transgenic mice.
Significance: objective testing of photosensitivity in humans and mice will provide new approaches to finding the underlying mechanisms, classification of photosensitivity, diagnosis and monitoring of new treatments.

15. SUBJECT TERMS
Photophobia, photodynia, photosensitivity, light sensitivity, traumatic brain injury, electromyogram, calcitonin gene related peptide (CGRP), trigeminal

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	23	

Table of Contents

INTRODUCTION

Two of the most prevalent problems reported by military personnel following traumatic brain injury (TBI) are photosensitivity and headache. Currently, better means are needed for diagnosing and treating post-traumatic light sensitivity and headache. The goal of this project is to establish a clinically translatable assay of photosensitivity to facilitate diagnosis and treatment of light sensitivity and headache. The clinically translatable assay will take advantage of a natural brain reflex, the photic-electromyogram (EMG), a reflex contraction of the eyelid muscles in response to light. The photic EMG is modulated by the thalamus and central sensory trigeminal pain center of the brainstem, which conveys light input to the facial muscles to elicit an eye blink and squinting response. We hypothesize that the hallmark of patients with photosensitivity is abnormal sensitization of the thalamus and brainstem trigeminal neurons to light input. This grant's objective is to show that the trigeminal and photic blink reflex to light, as measured by the photic EMG, is a valid surrogate for assessing central thalamic and trigeminal hypersensitivity as a cause for photosensitivity and headache, which can be treated. The specific aims of this grant are twofold: 1) to objectively characterize the photosensitive response in humans by recording the photic EMG in normal subjects compared to photosensitive patients and assess treatment with blue blocking lenses, and 2) to examine the photosensitive response in awake, un-anesthetized mice by recording the photic EMG in a genetic mouse strain that has trigeminal hypersensitivity and light aversion. The effect of injecting calcitonin gene related peptide (CGRP), the neurotransmitter modulating trigeminal neurons, and an antagonist, olcegepant, will be used to investigate a new medical treatment of photosensitivity in the mouse model. These studies will establish the foundation for future clinical diagnosis and treatment of photosensitivity.

BODY – RESEARCH ACCOMPLISHMENTS ASSOCIATED WITH APPROVED STATEMENT OF WORK FOR YEAR 3:

1) IRB and Animal Use submission

In Year 2, we were notified of approval of our human use protocol from the DOD Human Use Officer in the following memo (animal protocol approval occurred at the end of Year 1):

Classification: UNCLASSIFIED
Caveats: NONE

SUBJECT: Initial Approval for the Protocol, "Use of the PhotoElectromyogram to Objectively Diagnose and Monitor Treatment of Post-TBI Light Sensitivity," Submitted by Randy H. Kardon, MD, PhD, University of Iowa and Veterans Affairs Health Care System, Iowa City, Iowa, Proposal Log Number 11125001, Award Log Number W81XWH-11-1-0561, HRPO Log Number A-17005

The HRPO point of contact for this study is Lori J. Walther, Human Subjects Protection Scientist, at 301-619-2286/lori.j.walther.ctr@us.army.mil.

CARYN L. DUCHESNEAU, BS, CIP
Chief, Human Subjects Protection Review
Human Research Protection Office
Office of Research Protections
US Army Medical Research and Materiel Command

After receiving approval for the human use portion of the study, we have begun to identify patients with photosensitivity. We have access to a TBI database from the Iowa City VA Medical Center as a source for military-associated TBI. Patients with photosensitivity after TBI can also be identified as they are referred to the Iowa City VA Eye Clinic and seen by the PI (Randy Kardon MD PhD) in his VA and University of Iowa neuro-ophthalmology clinics. In addition, we can access the University of Iowa Hospital patient database by diagnosis allowing us to obtain an Excel spreadsheet listing patients with TBI and also those with photosensitivity as a diagnosis. We also have a list of normal subjects that we have used as research subjects for other studies and will be recruiting normal subjects from this pool, since their visual system has already been well characterized. Finally, we have also been recruiting migraine patients since they commonly report light sensitivity between headaches and we can recruit migraine subjects in the immediate 25-mile radius as subjects using email announcements and also the UIHC database by diagnostic category and patient location. Light sensitivity in migraine patients (between episodes) has relevance to the DOD, since post-traumaic migraine is very prevalent after TBI.

We already have had an approved IRB3 for a pilot study that has tested human EMG responses from the eyelid and forehead muscles as a function of light intensity. This had been performed in a limited number of normal subjects, migrainers and patients with traumatic brain injury who report light sensitivity. We are taking advantage of the pilot project experience to help refine the testing protocol and analysis of the EMG signals recorded that we will also be using as an outcome measure for the present DOD funded project.

2) Optimization of a novel method to objectively assess photosensitivity in humans and mice (months 1-36)

Task 1b. Integration of software with EMG recording to simultaneously record and analyze skin conductance, beat-to-beat variation in the electrocardiogram (ECG), and pupil

In Year 2 we have implemented software to calculate the area under the rectified EMG signal elicited from orbicularis and procerus muscles (muscles involved in blinking and squinting) obtained from both human and mouse measurements. This is a critical metric that we can now measure to reflect any sustained response, independent of the maximal RMS amplitude of EMG response to light. In our pilot data in mice and humans we found that the sustained response was very susceptible to the disease state being measured (light sensitivity), so this measurement will be an important outcome measure, which we can now quantify. During the 1st quarter of Year 3, we have applied this calculation metric to the transient phase of the EMG response to light in humans, and found that the peak EMG RMS values correlate well with the area-under-the-curve values as shown in Figure 1 and Figure 2.
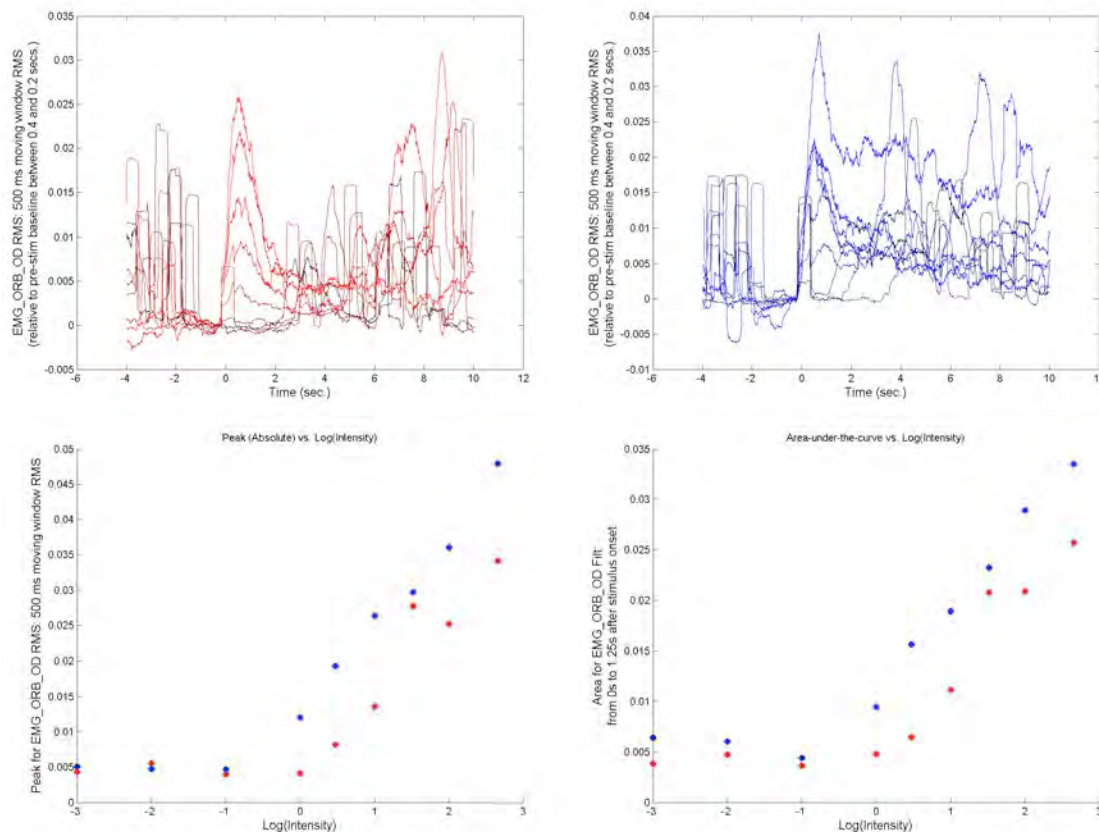

Figure 1. Example of orbicularis oculi EMG results from the right (OD) eye of a light-sensitive migraine patient tested recently. The RMS time series for the red light stimulus (left graph, red tracings) and blue light stimulus (right graph, blue tracings) are shown for increasing stimulus light intensities. The peak EMG RMS values at each stimulus intensity (between ~500ms to 1,000ms after stimulus onset) are shown in the bottom left graph (not adjusted for a pre-stimulus baseline), while the area-under-the-rectified-EMG-signal values (from 0ms to 1,250 ms after stimulus onset) are shown in the bottom right graph. Note there appears to be a greater EMG response as a function of log light intensity and the response is greater for blue light compared to red light, in which the intensities were photopically matched.
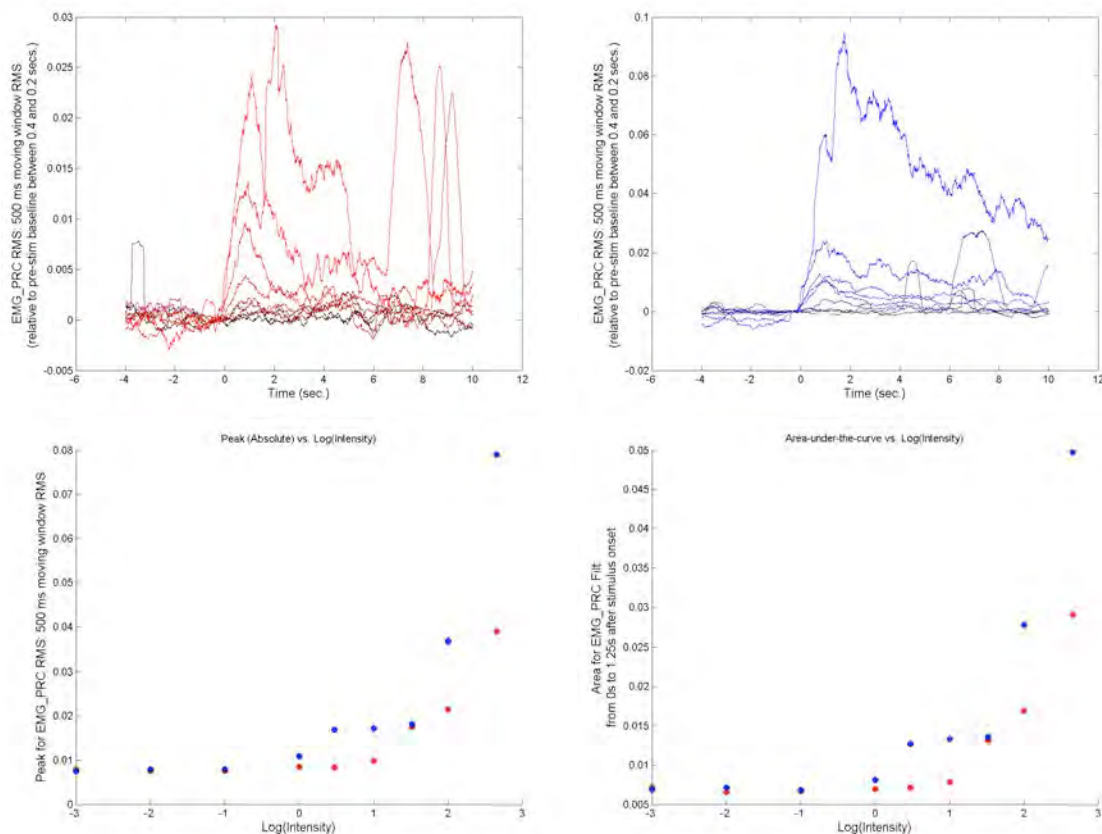
Figure 2. Example procerus/corrugator EMG results from a light-sensitive migraine patient tested recently. The RMS time series for the red light stimuli (left graph) and blue light stimuli (right graph) are shown for increasing stimulus light intensities. The peak EMG RMS values at each stimulus intensity (between ~500ms to 1,000ms after stimulus onset) are shown in the bottom left graph (not adjusted for a pre-stimulus baseline), while the area-under-the-rectified-EMG-signal values (from 0ms to 1,250 ms after stimulus onset) are shown in the bottom right graph. Notice how the EMG RMS and area-under-the-curve values increase exponentially for the brightest intensities, especially for blue. Lowering of the inner brow -- through the use of the procerus/corrugator muscles, produces extreme squinting, which may be an accessory response (last resort) to reduce peripheral retinal illumination without completely closing the eyelid given that additional orbicularis oculi activity will tend to cause complete eyelid closure at the brightest intensities.

During the 1st quarter, we have also implemented a Matlab GUI-enabled tool for reviewing and visualizing video and time series data within a unified and user-specified framework. Video and time series data are synchronized based on timestamps. The GUI enables the user to playback, rewind, etc. recorded data, and easily navigate to any timeslice by clicking in any of the linked graphs, as well as jump to any specific stimulus epoch. The video frame that is displayed for each video stream is calculated on the fly and based on the current timestamp. The user has the ability to specify the number of graphs, as well as how each time series is to be assigned to any Y axis (both left and right for each graph), and can use the mouse-controlled crosshair to read off the x, y1 (left), and y2 (right) coordinates on any graph. The user can also record the GUI window as a standard video file in order to create movie clips for presentations, etc. This tool is used for reviewing data from both our human and mouse studies, simply by specifying the relevant input data sources and GUI layout parameters for each type of data

7

recording session. An example of data from our human experiment setup is shown in Figure 3, and data from an animal experiment is shown in Figure 4. Through the use of the GUI tool, we were able to qualitatively compare the increase in EMG RMS level to changes in the appearance of the eyelids and other facial features with increased stimulus intensity. These results are shown in Figure 5.
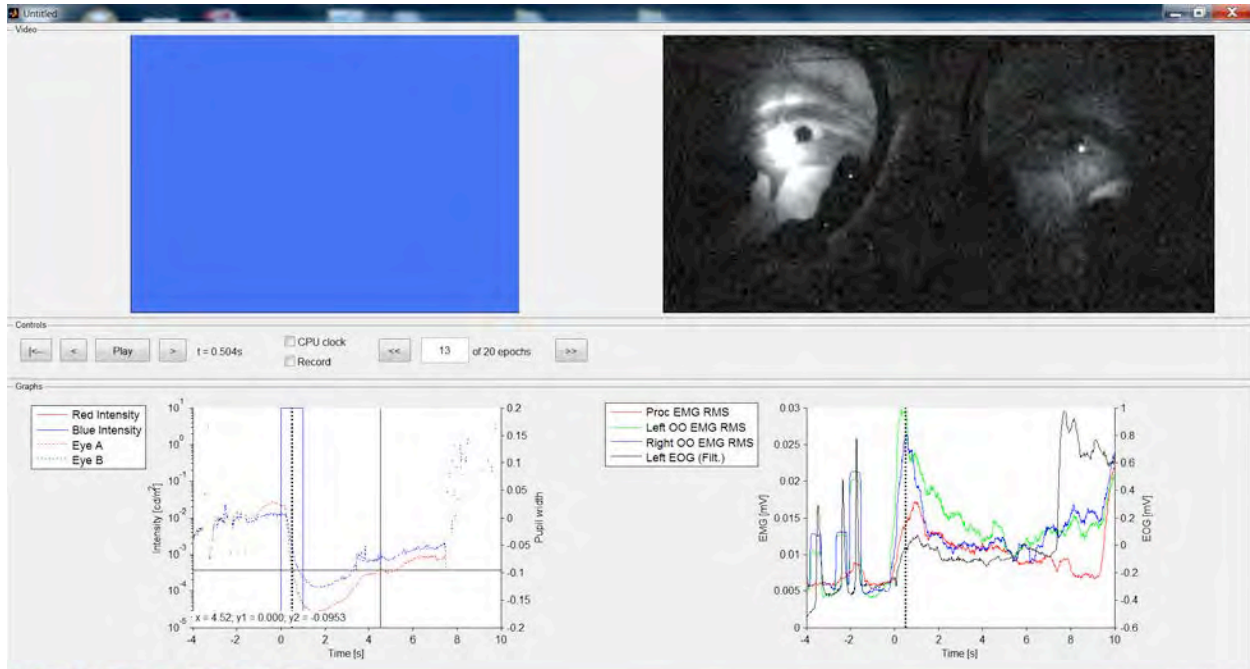

Figure 3. A screenshot of the Matlab GUI populated with example data from a human subject. The experiment setup consisted of a Diagnosys Ganzfeld bowl to present 1-second red and blue light stimuli, while an Arrington eye frame mounted eye tracker with miniature video cameras was used to collect pupil data, and a wired Biopac system collected EMG, EOG, etc. data. In this example, data are displayed for t=0.504s after stimulus onset (as indicated by the vertical black dotted line in each graph), at about the time when the EMG RMS and EOG values peak, as can be seen in the graph on the lower right hand side. Stimulus and pupillometry data are shown in the graph on the left hand side and in this case a one second blue stimulus was given (rectangular blue step in tracing). The left video frame shows that a blue stimulus is presented in the Diagnosys bowl at this time instance, and the right video frame shows the subject's face as seen from the built-in IR camera inside the bowl pointed at the subject. At this video frame, captured at the peak of the EMG response, it can be observed that the eyelids are partly squinted closed in response to the bright blue light stimulus, coinciding with the EMG tracing in the lower right graph.
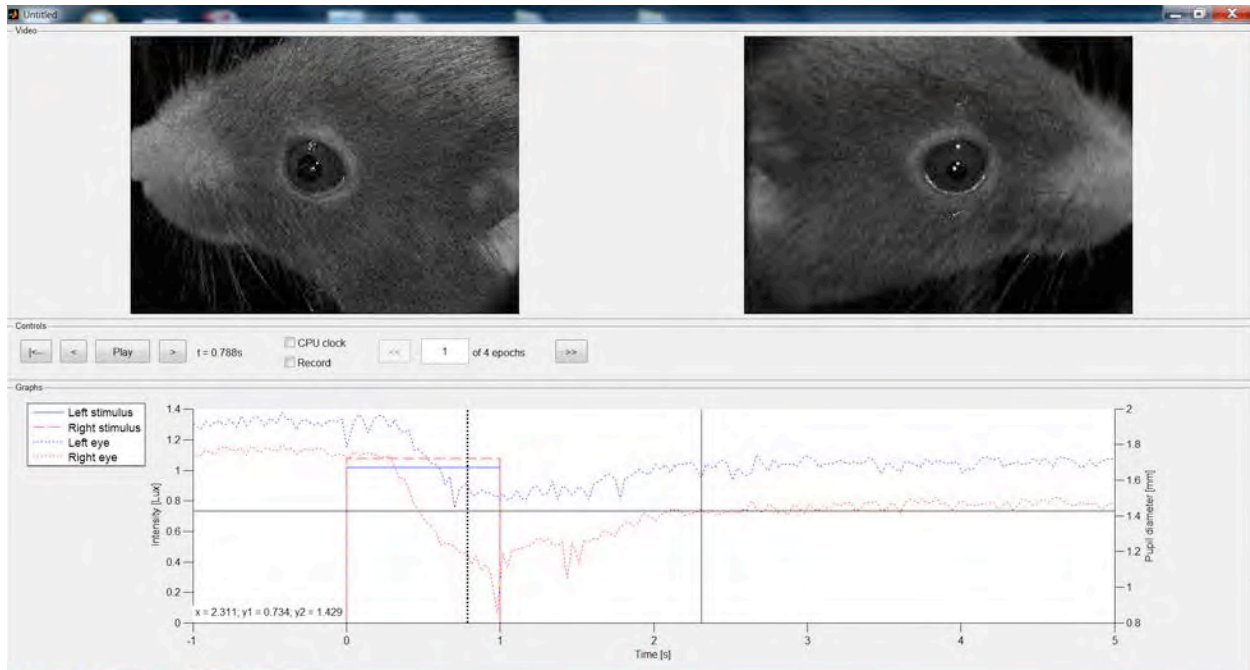
Figure 4. A screenshot of the Matlab GUI populated with example data from a mouse subject. The experiment setup consisted of a Neuroptics A2000 stimulus presentation and pupillometry system to present 1-second red and blue light stimuli and for measuring pupil diameter simultaneously. In this example, data are displayed for t=0.788s after stimulus onset (as indicated by the vertical black dotted line in the graph). Stimulus and pupillometry data are shown in the graph. The left video frame shows the left side of the mouse, and the right video frame shows the right side of the mouse, as seen from IR cameras of the A2000 system. Note that the stimulus in this example was given to the right and left eyes but the right pupil constricts more than the left pupil due to decreased input to the left eye from a blast injury, due to greater decussations in the afferent and efferent pupil light reflex pathway in a mouse compared to a human. A similar recordings are also made for recording the EMG in mice with chronically implanted electrodes in the orbicularis muscle in the photosensitivity experiments, shown in subsequent figures.
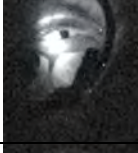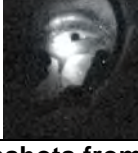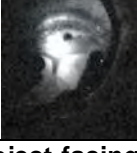
Log Intensity (log cd/m2)	Red Light Stimuli Increasing Intensities		Blue Light Stimuli Increasing Intensities	
-3				
-2				
-1				
0				
0.5				
1				
1.5				
2				
2.6				

Figure 5. Snapshots from the video stream of the subject-facing camera (from the data set used in Figures 1, 2, and 3), which are time synchronized with the EMG recording, showing the position of the upper and lower eyelids at the instant of the peak EMG RMS value for each combination of stimulus color and intensity. Notice how increased squinting -- due to increased orbicularis oculi

EMG activity as shown in a previous figure -- is clearly visible as a reduction in the size of the eyelid fissures with increased stimulus intensity. Eyelid fissures also appear smaller under the blue light condition in comparison to the fissure sizes for the red light condition at the same photopically matched stimulus intensity. These observations support the notion that the utility of videography and image analysis -- for tracking changes in eyelid position and eye brow shape -- could be used as a substitute method for "measuring" EMG activity in future, and might open up significant telemedicine and at-home testing possibilities using just video based systems to assess light sensitivity.

Based on the results shown in Figure 5, snapshots from a subject-facing video camera, taken at the instant of the peak EMG RMS value for each combination of stimulus color and intensity, show how increased squinting -- due to increased orbicularis oculi EMG activity -- is clearly visible as a reduction in the size of the eyelid fissures with increased stimulus intensity. Eyelid fissures also appear smaller under the blue light condition in comparison to the fissure sizes for the red light condition at the same stimulus intensity. We have therefore made the argument that these observations support the notion that the utility of videography and image analysis -- for tracking changes in eyelid position and eye brow shape -- can be used as a substitute method for "measuring" EMG activity in future, and might open up significant telemedicine and at-home testing possibilities using mobile video devices.

To this end, we have acquired and tested an off-the-shelf commercially available software solution during the 2nd half Year 3, which implements a computerized version of the Facial Action Coding System (FACS). In short, FACS is a system to taxonomize human facial movements by their appearance on the face. Movements of individual facial muscles are encoded from slight and instant changes in facial appearance. Although FACS is an index of facial expressions, and does not strictly provide any bio-mechanical information about the degree of muscle activation per se, the system attempts to estimate the fundamental actions of individual muscles or groups of muscles, called action units (AU), as well as estimate intensity scores of each AU on a 5-point scale as Trace, Slight, Marked or Pronounced, Severe or Extreme, and Maximum. Recent advances in image analysis coupled with increased computer processing power have yielded software solutions able to automatically estimate AU scores, using video data collected from a basic subject-facing camera, such as a webcam. Based on the objectives of this research effort, we have identified a list of AUs that are significantly correlated to the EMG activity of the orbicularis oculi, corrugator and procerus muscles we are currently recording during experiments:

AU1   Inner Brow Raiser   frontalis (pars medialis)
AU2   Outer Brow Raiser   frontalis (pars lateralis)
AU4   Brow Lowerer   depressor glabellae, depressor supercilii, corrugator supercilii
AU5   Upper Lid Raiser   levator palpebrae superioris, superior tarsal muscle
AU6   Cheek Raiser   orbicularis oculi (pars orbitalis)
AU7   Lid Tightener   orbicularis oculi (pars palpebralis)
AU41   Glabella Lowerer   Separate Strand of AU 4: depressor glabellae (aka procerus)
AU42   Inner Eyebrow Lowerer   Separate Strand of AU 4: depressor supercilii
AU43   Eyes Closed   Relaxation of levator palpebrae superioris
AU44   Eyebrow Gatherer   Separate Strand of AU 4: corrugator supercilli
AU45   Blink   Relaxation of levator palpebrae superioris; contraction of
orbicularis oculi (pars palpebralis)
AU46   Wink   orbicularis oculi

During the 3rd quarter, we have collected several test datasets to investigate the utility of FACS in more detail. Instead of recording EMG data with surface electrodes attached to the subject's face, we used the camera built into the Diagnosys light stimulus bowl to record an infrared video

of the subject's facial expressions in response to our standard testing protocol, which comprises of a sequence of red and blue light flashes of increasing intensity. Subsequently, the video was fed through the FACS software to estimate the different AU metrics for each video frame. After synchronizing the video frames with the Biopac recording of stimulus onsets (sensed as a TTL signals from the Diagnosys bowl), we were then able to playback and study changes in the reported level of FACS AU metrics in response to the different stimuli, as shown in Figure 6. Please note that the image quality of the built-in Diagnosys camera was low due to pixel noise and low contrast, which contributes significantly to the noisy FACS AU values evident in the figure. However, even though the camera resolution is only 640x480 pixels, it provides sufficient pixel density based on the minimum requirement of the FACS software of having at least 120 pixels spanning the width of the subject's face (i.e., ear to ear). We are currently working with Diagnosys to retrofit a higher resolution camera and upgrade the infrared illumination system inside the bowl in order to record higher quality videos and to record a larger field of view, capturing the entire face in response to different light levels.
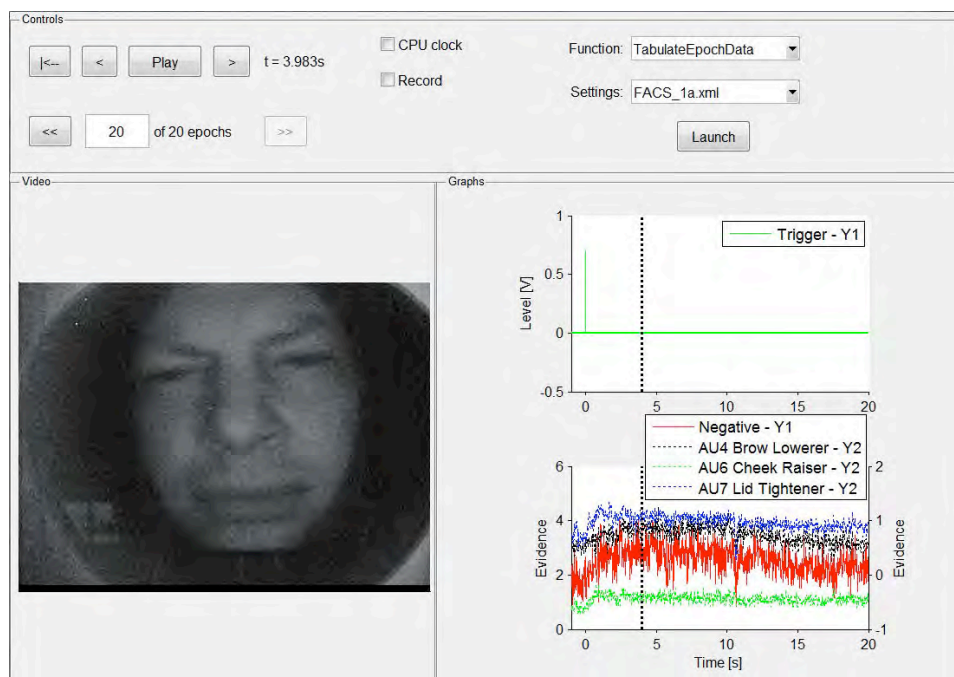
Figure 6. A screenshot of the Matlab GUI tool populated with data from our first FACS test run, showing how the video analysis of the facial muscles can provide quantitative measures of contraction in response to light. The experiment setup consisted of the Diagnosys bowl to present a sequence of red and blue light flashes of different durations and intensities, while the Biopac system recorded TTL pulses sent from the bowl to signal the onset of each flash, and video of the subject's face was recorded from a camera inside the bowl. In this example, data are displayed at t=3.983s following the onset of the final 10 s 400 cd/m^2 blue flash (as indicated by the vertical black dotted line in each graph). Stimulus-related data are shown in the top graph, while time series of different FACS metrics synchronized to the stimulus data are shown in the bottom graph. The video frame shows the subject squinting in response to the bright blue stimulus, which correlates with increases in the level of the AU4, AU6, and AU7 metrics, as well as the level of Negative affect (which represents a combination of different negative emotions, such as disgust, fear, anger, etc.).

Stimulus-response curves for three subjects tested with the FACS method described above are shown in Figure 7. On average, the level of the AU7 metric is higher for blue stimuli when compared to the response for a photopically-matched red stimulus, which correlates with the EMG findings we have reported previously under the same test conditions.
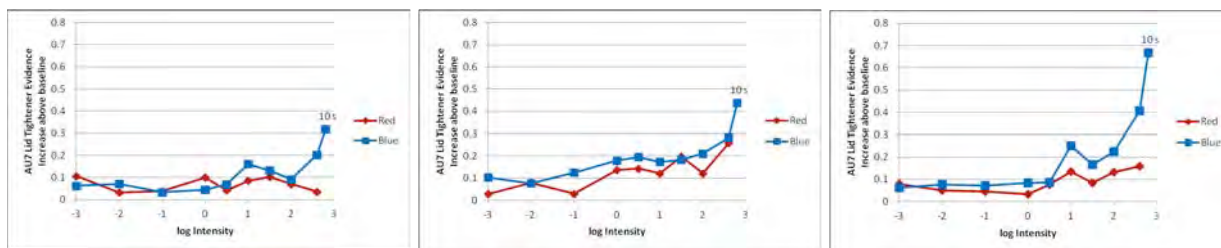
Figure 7. Stimulus-response curves of facial muscle contractions in response to increasing log light intensity for 3 different (normal) test subjects (average for 2 test repetitions for each subject). The graphs depict the increase in the level of the AU7 (orbicularis muscle) metric above baseline across a range of red and blue stimulus intensities (reported in log cd/m^2). All stimuli are 1 s in duration, except for a single 400 cd/m^2 blue stimulus with a 10 s duration, as indicated in each graph. The subject on left has brown eyes, while the other 2 subjects (middle and right) have blue eyes.

Based on the encouraging results from the FACS tests, we have submitted an Invention Disclosure to the University of Iowa Research Foundation, titled " Use of Facial Action Units to Diagnose and Treat Light Sensitivity", with an eye on patenting the concept. Here follows an excerpt of the disclosure:

"The major focus of this invention is to provide a novel, robust and easy to obtain biological biomarker to diagnose and treat photosensitivity (photophobia). Photosensitivity can occur in association with a variety of conditions, including migraine, traumatic brain injury, albinism, retinal dystrophies/degenerations such as achromatopsia, in association with some brain tumors, and after meningitis. It can be debilitating in daily life, interfering with reading, working, driving. Currently, there is no objective method for accurately diagnosing photosensitivity other than a patient's subjective complaints, which also makes it difficult to assess the effectiveness of any treatment. This invention will solve this problem by providing, for the first time, a quantitative analysis of the light-induced activation of facial muscles. The Facial Action Coding System (FACS) was originally derived from observable facial muscle movements in parallel with EMG recordings from the corresponding muscles. More recently, this technology has been translated to analysis of facial features from a picture or frame of a video recording to provide a quantification of the activation level of at least 19 different facial action units. The most popularized application of FACS has been to combine facial action units to score and compare the emotional responses of subjects to a variety of visual scenes, which evoke fear, anxiety, disgust, anger, etc. In this invention, we will take the FACS application a giant step further, by using a well-defined sequence of visual stimuli, increasing in light intensity, to evoke both reflex and emotional facial responses to stimuli in normal subjects and in patients with disabling photosensitivity. To our knowledge no one has ever applied the FACS approach to objectively characterize light sensitivity. In our prior research effort, we demonstrated that the light induced electromyogram (photic-EMG) of the orbicularis and corrugator/procerus muscles was hyper-activated by light in patients with photosensitivity compared to control subjects."

Task 1c. EMG recording from chronically implanted electrodes in the mouse will be developed using the DSI wireless system of data transmission of biopotentials.

During Year 3, we have engaged DSI to add video recording capabilities to our existing DSI wireless EMG system, based on our thinking that a video record of the mouse's response to light and air puff stimuli provides significant additional proof beyond the EMG record of whether the mouse is either blinking or squinting in response to a stimulus. During the first 3 quarters,

13

we have spent a significant amount of effort working with DSI to iron out several issues with their video recording solution. DSI provided us with a video-enabled loaner system for testing out the utility of the video recording system before purchasing the additional hardware components and software licenses for adding video recording capabilities to our existing DSI system.

One of the most important issues we had to find a solution for relates to unreliable and inaccurate synchronization of the recorded video with the EMG data. To complicate the matter, video is recorded in the DSI software in a variable frame rate file format, with incorrect time offsets, while the recording during an experiment might be broken up into several files at random times, together with the loss of an unknown number of video frames over several seconds while the DSI software is closing one video recording file and opening a new file. Fortunately, the camera used by the DSI system is a home security type camera, which can be set up to synchronize its clock with a time server on the network, and can be also programmed to overlay the time onto each video frame. However, in order to read the timestamp from each frame, we had to implement optical character recognition (OCR) as part of our Matlab analysis routines.

We have previously reported that we had implemented a Matlab GUI-enabled tool for reviewing and visualizing video and time series data within a unified and user-specified framework. This tool is used for reviewing data from both our human and mouse studies, simply by specifying the relevant input data sources and GUI layout parameters for each type of data recording session. During the 3rd quarter, we have adapted the tool for use in reviewing and analyzing the video and EMG data collected recently for several implanted mice. An example of the video and EMG data collected recently for an implanted mice is shown in Figure 8.
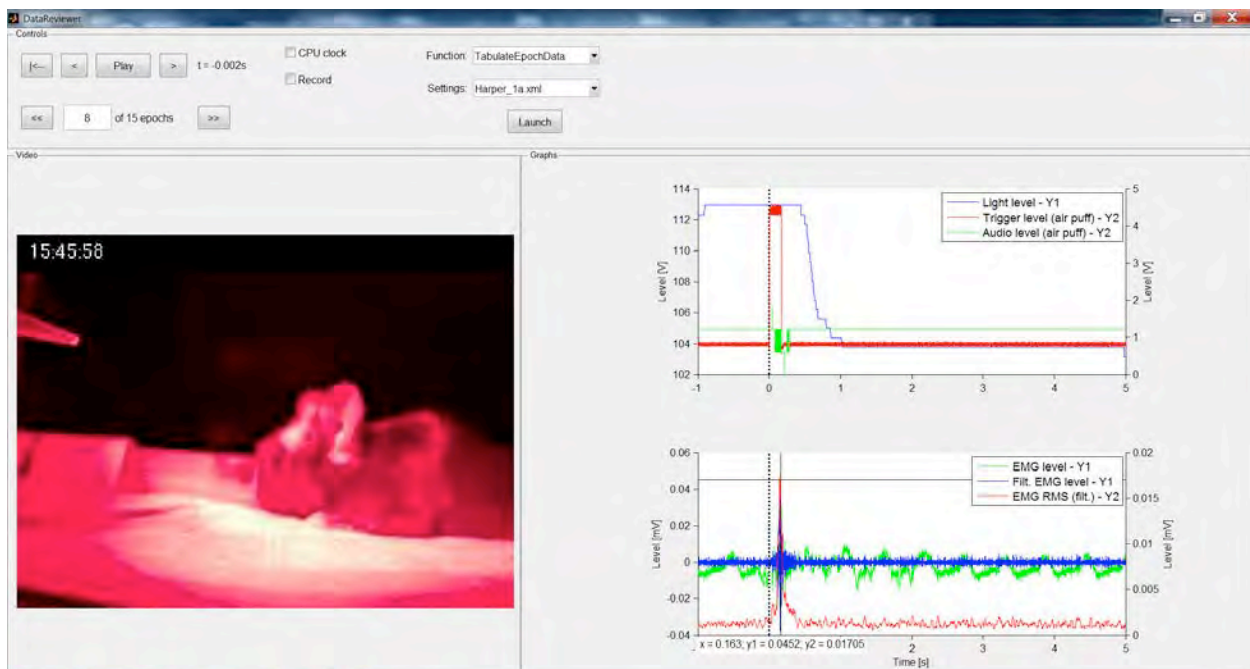
Figure 8. A screenshot of the Matlab GUI tool populated with data from an instrumented mouse. The experiment setup consisted of our electronically-activated air puff delivery system to present bursts of air puffs, while the wireless DSI system collected EMG, air puff audio, background light intensity, etc. data, as well as recorded video from the camera pointed at the mouse. In this example, data are displayed for t=0.002s before stimulus onset (as indicated by the vertical black dotted line in each graph). The crosshair is located at t=0.163s, which is about the time when the

EMG RMS value peaks, as can be seen in the bottom graph. Stimulus-related data are shown in the top graph. The video frame shows the mouse and the pipette tip used to direct the air puff at the mouse. Note the timestamp overlaid onto the video frame, which is read with our OCR routine and used to synchronize the video with the recorded EMG data.

In the last quarter, we have decided to abandon the DSI-sourced video recording solution based on unresolved issues, unstable performance, and pricing. However, based on lessons learned with the DSI video solution as well as the fact that the DSI system is based on a widely-used home security video system, we were able to purchase off-the-shelf components to assemble our own in-house video recording solution. We have configured the system to record from 3 video cameras, of which one is the built-in infrared camera inside the Diagnosys Ganzfeld light bowl, described below. Over the past few months, we have tested the system extensively, and we are happy to report that it is performing according to our requirements.

For our photic testing in mice during Years 1 and 2, we had been using an LED array that ranges in white light from 0 to 27,000 lux intensity. In initial studies this device has been sufficient. However, in an effort to further refine the photic testing and to reduce excessive electromagnetic noise produced by the LED array electronic components, we have acquired a Ganzfeld light bowl from Diagnosys LLC during the past year as shown in Figure 9. The Diagnosys Ganzfeld bowl is the same model as the one used in our human studies, and allows us to control the intensity, duration, and wavelength of light administered to the mouse while recording subsequent EMG signals and video of each event. The system includes a control unit with desktop PC to precisely drive the Ganzfeld bowl stimulus. We have been using the Ganzfeld bowl stimulus exclusively in the mice studies over the past year.
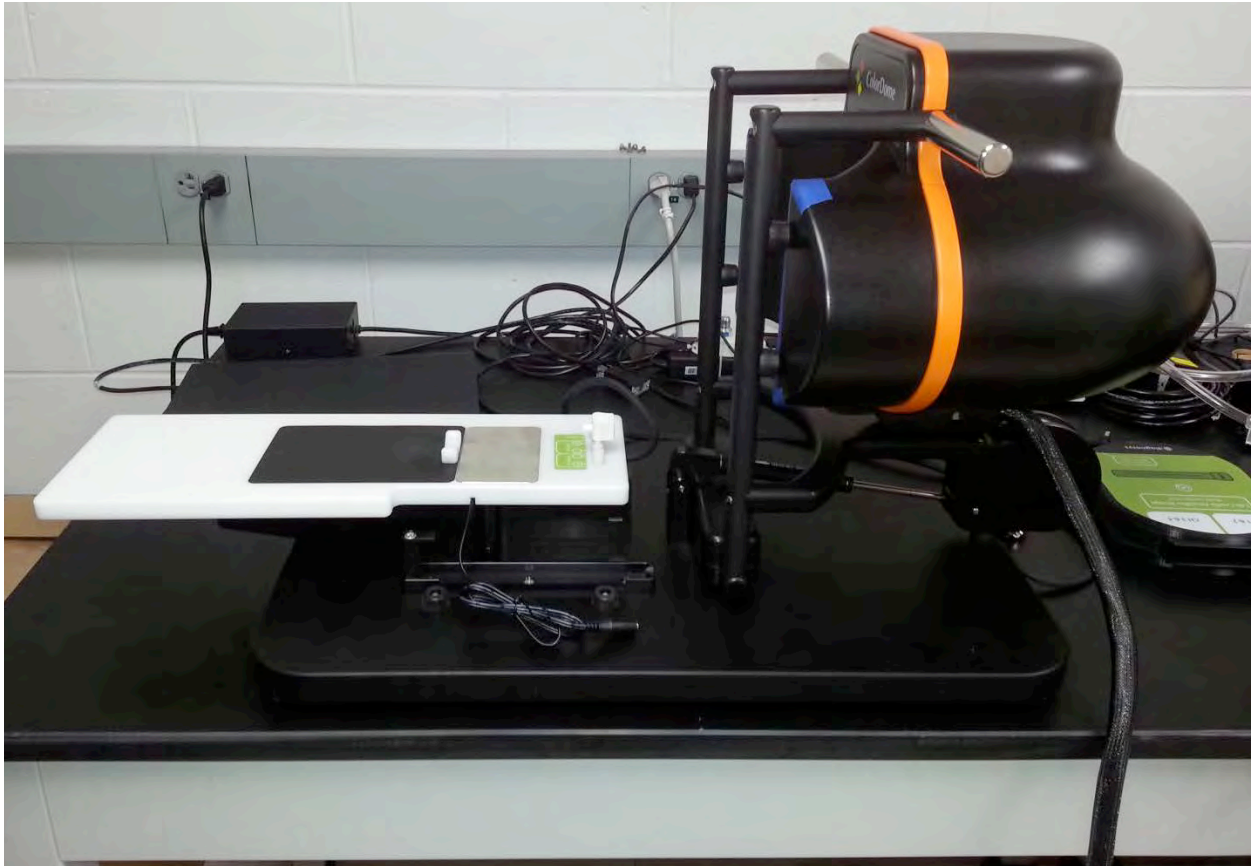
Figure 9. A look at the Diagnosys Ganzfeld bowl which provides the light stimulus to the mice. The bowl has been made to hinge into position over the mouse to provide wide field visual stimulation, in parallel to human experiments.

Task 2 (Year 2). In normal humans without photosensitivity, collect and define the normative range of values for light induced EMG, pupillary light reflex, skin conductance, and ECG in response (months 13-24):
2a. Collect data on 50 normal subjects with optimized protocol for testing light induced outcomes as in Task 1; 25 subjects without history of TBI and 25 post TBI normals (months 13-24)
2b. Analyze pupil light reflexes, skin conductance, ECG, and EMG (months 13-24)

We have not been able to start data collection due to the following reasons:
- The development of the hardware and software analysis tools for assessing the main outcome measures (activation of facial muscles in the forehead and around the eye, pupil light reflex, skin conductance, and heart rate variation) has taken longer than originally anticipated. Since the entire project hinges on these outcome measures and being able to efficiently test patients in a short period of testing and analyze the results in real time, the project time line has become longer.
- Due to clinical space management issues and construction, there was a delay in securing an adequate room for testing of subjects that was in close enough proximity to the eye clinic to be able to test the light sensitive subjects needed to satisfy entry criteria into the study. A room has been reconfigured and equipment has now been moved into that space so that subject recordings can now be collected as original proposed for testing of patients.

16

- We have recently discovered that the activation of the facial muscles by light can be effectively assessed by recording a video of the change in facial muscles at different light levels. Here we have recently taken advantage of available methods of quantifying facial muscle contractions by analyzing the "facial action units" for individual facial muscles, derived from the Facial Action Coding System (FACS). This is a method of quantifying small movements of facial muscle activity that will eventually supercede the need to record electrical responses from the squinting and blinking muscles. We are presently modifying our recording system to include the video recording as this has very powerful translational potential for the DOD, VA and medical care. This would allow remote monitoring and assessment of light sensitivity using the video assessment system. We now consider this a critical outcome measure to incorporate into the testing protocol for enrolled subjects.

Based on these reasons, we are in the process of submitting an EWOF to complete the testing in the next 2 years.

Task 4 (Year 2). In normal littermate mice and in mice rendered photosensitive (genetically altered to over-express the receptor for (CGRP), compare the EMG response to light with intra-ventricular vehicle injection vs CGRP (months 13-24):
4a. Record and analyze eyelid EMG responses to light in normal littermate control mice of the hRAMP1 strain, comparing vehicle with intra-ventricular CGRP (months 13-24).
4b. Record and analyze eyelid EMG responses to light in genetically altered hRAMP1 photosensitive mice, comparing vehicle with intra-ventricular CGRP (months 13-24).

Anesthesia:
During the 1st quarter we made a switch from ketamine/xylazine to isoflurane for anesthesia. Currently, we use a concentration of 5.0% isoflurane and 1.0% oxygen to induce the mouse while a maintenance concentration of 2.0% isoflurane and 1.0% oxygen is used during surgery. Compared to ketamine/xylazine, isoflurane is much more effective at fully anesthetizing the mice as well as providing a speedy recovery time (~10 minutes). The method is also much more effective at keeping mice anesthetized for longer surgeries such as this. With ketamine/xylazine, mice larger than ~25 g were difficult to fully anesthetize and thus were omitted from use. Using isoflurane, larger mice can and have been effectively used for implantation. Their larger size helps accommodate the implant more so than smaller mice.

Implantation Surgery:
Standard method: Instead of making one large incision over the top of the mouse's skull, two smaller incisions are made: one small midline incision just superior to the eyes and one midline incision between the scapulae. The skin over the top of skull remains uncut. The caudal incision is just large enough for the transmitter to pass through while the rostral incision is just large enough for the insertion of the electrodes into the orbicularis oculi. A trocar and sleeve are used to tunnel between incisions and thread the electrode wires. The two incisions are sutured using dissolvable 7-0 coated Vicryl suture to prevent further irritation. The healing process is alleviated by not completely incising the area over the skull. The tunnel opened up by the trocar keeps the wires in place more effectively as well as provides a smaller area for the adhesive to cover, thus providing the possibility for ICV injections. The wires are not showing no signs of being excised from the mouse, providing additional time for viable testing.

Improvements: During Year 3, we became aware of the need to keep the electrodes apart at a short fixed distance to minimize the amount of crosstalk the electrodes can pick up from other muscles of the body, while at the same time ensuring that the electrodes don't touch and short circuit. Additionally, the goal is to anchor the electrodes in the orbicularis oculi while exposing a

minimal amount of electrode surface area. Utilizing the electrode's structure, which is a tightly wound coil, we have been able to tie two separate, non-conducting, non-dissolvable suture anchors between the terminal ends at two locations. By tying the anchors between two coils, the electrodes cannot move independently from each other. Also, with the tie between the electrodes holding firmly, the suture impedes the electrodes from moving in the tunnels made to place them. One location is just distal to the original scaffold (please see Figure 10) to keep the electrodes from sliding further out. The other location is at the surface of the skin to keep the electrodes from retracting under the skin. A final single suture is used to hold the outside anchor close to the skin surface so the conscious mouse cannot use a digit to displace the implant.
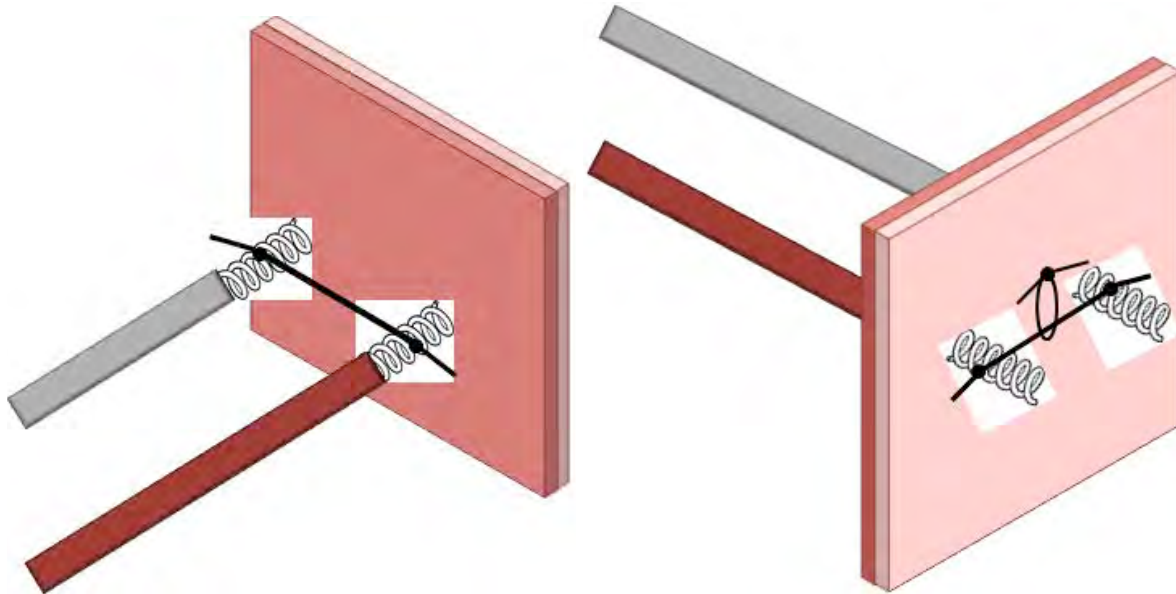

Figure 10. Rendering of the sutures used to anchor the electrodes in place. The sutures are non-dissolvable and remain with the mouse the duration of the implant. A minimal amount of glue is added to each knot to keep the suture secure. All other use of glue in the implantation process has been eliminated.

This implantation method has greatly increased the viable testing time per mouse. Before, the time window during which reliable data could be recorded was on average one to two weeks, but with the scaffolding method we are now able to gather data from an implanted mouse for seven weeks. Additionally, EMG signals from the implanted mice are cleaner and the movement artifacts observed when using glue to secure the electrodes have been significantly reduced.

Experiment Protocol:
With our initial setup, mice were allowed to freely roam during experimentation. It was observed that majority of the EMG recorded was from surrounding muscle groups. EMG resulting from the orbicularis oculi was difficult to discern. In order to more accurately measure EMG from the orbicularis oculi, we constructed a mouse restraining box during the last quarter. The acrylic box, shown in Figure 11, allows the mouse to freely move its head in all directions while inhibiting overall movement. This minimizes surrounding EMG activity, ensures the mouse is receiving the full light stimulus, and can easily be recorded via camera to confirm observations and link EMG with visible response.

Figure 11. As part of the current testing protocol, the mice are gently restrained using a custom made acrylic box that allows video recording of blink activity with our in-house video recording system, described earlier. Light stimuli are delivered using the Diagnosys ColorDome light bowl after a dark adaptation period of 15 minutes, while the DSI plate reader below the acrylic mouse holder is used to transfer the EMG signals from the mouse wirelessly to a computer.

Results:

Video data recorded with our current setup shows that, while all mice keep their eyes open in the dark, our transgenic mice close their eyes in the light after administration of CGRP. The control mice do not exhibit this eye closure. Surprisingly, our EMG recording data show an overall decrease in EMG activity after CGRP administration, which is explained in the video analysis by almost complete eye closure . This is opposite of what was observed in our earlier experiments, which showed a marked increase in EMG activity after CGRP due to squinting, but not complete eye closure. In fact, we have been observing a simple eye closure (please see Figure 12) and reduction in eyelid and locomotor movement with CGRP in bright light. This behavior also matches human migraineur behavior in that increased photosensitivity, and any movement and muscular contraction exacerbate pain, causing the subject to seek out a dark environment, while trying to stay motionless (Figure 13 and Figure 14).
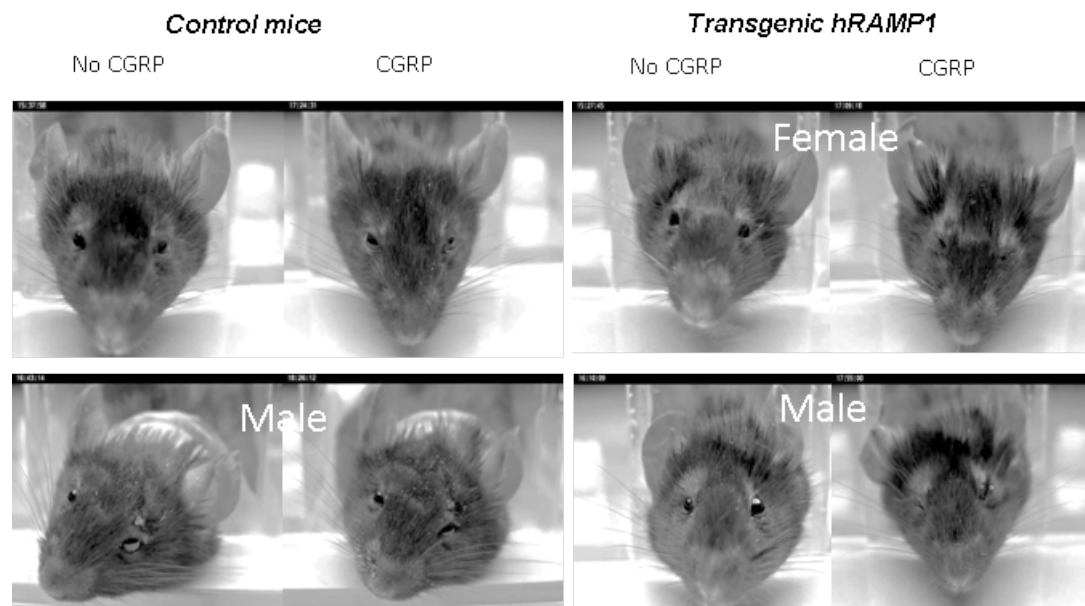
Figure 12. CGRP-induced eye closure in conscious mice. Control mice (left panels) and transgenic hRAMP1 (right panels) were videotaped before (left) and after (right) administration of CGRP (0.5mg/kg, IP) during a blue light stimulus. Note that marked eye closure in response to CGRP is seen only with the transgenic mice (far right panel).
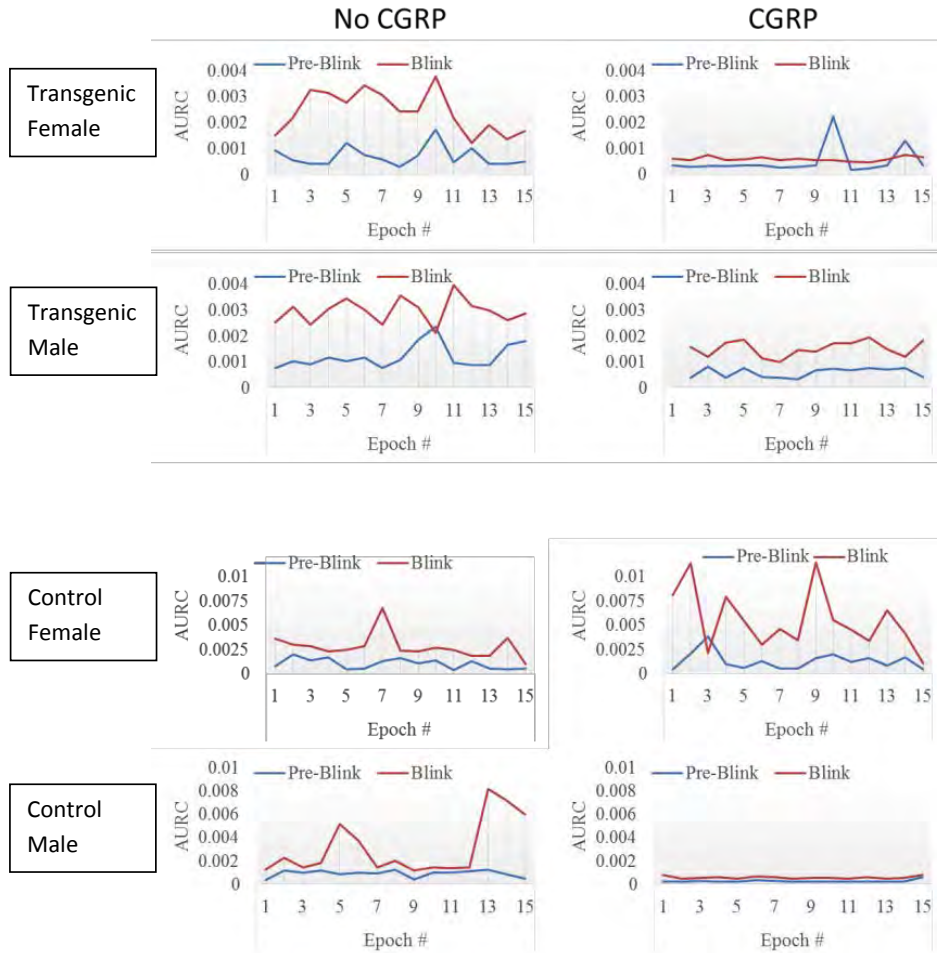
Figure 13. Blink-induced EMG activity of transgenic and normal mice. A blink was induced by a gentle air puff directed at the eye and confirmed with the recorded video. Pre-Blink refers to EMG signal calculated as area under the rectified curve (AURC) 500ms directly before the blink onset and Blink refers to the AURC 500ms after blink onset. Blinks were induced while the mouse was exposed to light stimuli (each stimulus consists of a 5 s flash followed by 15 s darkness) of increased light intensity ranging from 0.01% (Epoch #1) to 100% (Epoch #13) of maximum light dose. The duration of the light stimulus for Epoch #14 was 10 s and for Epoch #15 the duration was 30 s. Panels on the left show the EMG AURC signals before CGRP administration, and panels on the right correspond to measurements taken 20 min after CGRP (0.5 mg/kg, IP) administration. Note both transgenic and control mice showed increased blink-induced EMG activities, which were apparently not affected by the intensity of the light stimulus. After CGRP administration, the magnitudes of the blink-induced EMG activity for both transgenic mice and the male, but not female, control mouse were decreased, due to eye closure (see Figure 12).
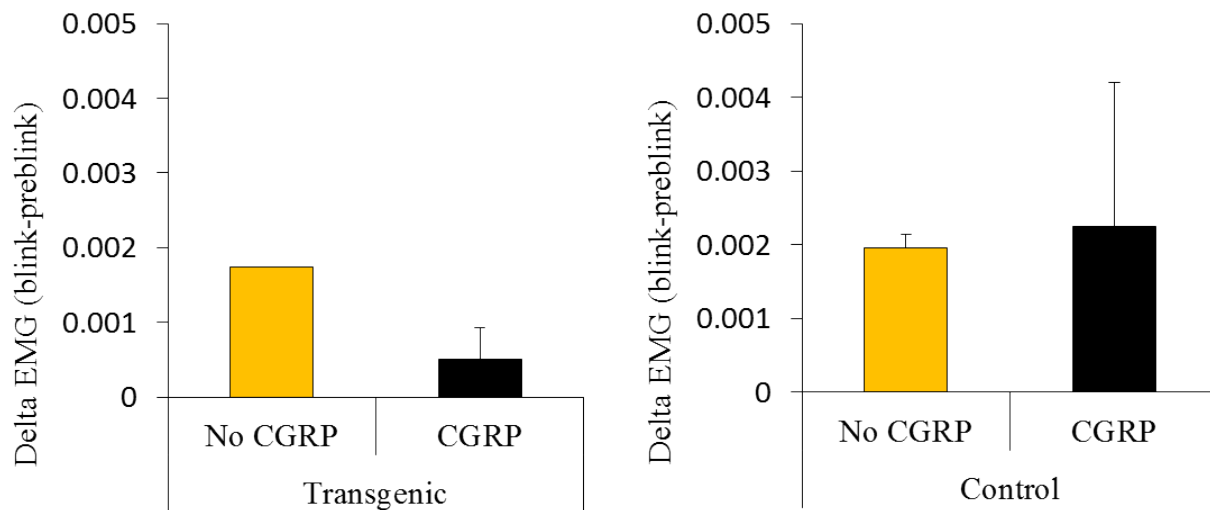
Blink-induced EMG signal

Figure 14. Blink-induced EMG activity. The difference between EMG blink activity and pre-blink activity (i.e., Delta EMG activity) is smaller for the transgenic mice after CGRP administration compared to the No CGRP condition due to eyelid closure, while control mice did not show an overall decrease in EMG blink activity between the two conditions on average.

KEY RESEARCH ACCOMPLISHMENTS (SUMMARY)

- We have found that for the transient phase of the EMG response to light in humans, the peak EMG RMS values correlate well with the area under the rectified EMG signal values.
- Based on Facial Action Coding System (FACS) metrics extracted from video recordings, we have shown that videography and image analysis -- for tracking changes in eyelid position and eye brow shape -- can be used as a substitute method for "measuring" EMG activity unobtrusively.
- We have implemented a Matlab GUI-enabled tool for reviewing and visualizing video and time series data within a unified and user-specified framework, and used it extensively for data analysis in both human and animal studies.
- Based on the encouraging results from the FACS tests, we have submitted an Invention Disclosure to the University of Iowa Research Foundation, titled " Use of Facial Action Units to Diagnose and Treat Light Sensitivity", with an eye on patenting the concept.
- For the studies with mice, we have acquired and integrated a Diagnosys Ganzfeld light bowl to match the fine-grained control to present light stimuli available for our human testing.
- We have designed and implemented a multi-camera recording system to provide video recordings of mouse behavior synchronized to the EMG recorded from electrodes implanted in the mouse orbicularis oculi muscle.
- In contrast to earlier results, we have observed an overall decrease in EMG activity after CGRP administration, especially for hRAMP1 transgenic mice.

REPORTABLE OUTCOMES

Presentation of the influence of eyelid position and the photic blink reflex upon the pupil light reflex as a poster at the Association of Research and Vision in Ophthalmology (ARVO), Ft. Lauderdale, FL May 2014.
.
Presentation of preliminary results as Visiting Professor, Washington University, Department of Ophthalmology, St. Louis, MO, November 2013.

Cyber Seminar, sponsored by DOD and VA on Photosensitivity after Traumatic Brain Injury (TBI): Mechanism, Diagnosis and Treatment, October 13, 2013.

CONCLUSIONS

The research work that we are carrying out has important implications for the greater public good, in addition to its military relevance. Light sensitivity and migraine headaches following traumatic brain injury are the two most commonly reported symptoms in military personnel exposed to direct trauma to the brain or indirectly from blast injury. Similar symptoms can also occur in the civilian population from TBI resulting from motor vehicle accidents and also from head injury due to contact sports at both the school and professional level. At present there are no biological markers or tests that can be used to objectively diagnose and monitor treatment of photo-- sensitivity or migraine headaches. This would be the first research to facilitate investigations of the mechanisms in humans using controlled, photic stimuli with monitoring of physiological reflexes in response to the light stimuli. In order to accomplish this task, it is required that a sophisticated software and hardware integration be in place to accurately measure light evoked reflexes that can be used in research and in a clinical setting. In addition, adding the capability of studying the photic EMG in conscious mice will provide an important scientific platform upon which to use genetic and drug investigations on the mechanism of light sensitivity and migraine and new treatments.

REFERENCES – a literature search was performed to update the previous literature on photophobia associated with TBI and yielded the following relevant references:

1: Ciuffreda KJ, Ludlam DP, Thiagarajan P, Yadav NK, Capo-Aponte J. Proposed objective visual system biomarkers for mild traumatic brain injury. Mil Med. 2014 Nov;179(11):1212-7. doi: 10.7205/MILMED-D-14-00059. PubMed PMID: 25373043.

2: Truong JQ, Ciuffreda KJ, Han MH, Suchoff IB. Photosensitivity in mild traumatic brain injury (mTBI): a retrospective analysis. Brain Inj. 2014;28(10):1283-7. doi: 10.3109/02699052.2014.915989. Epub 2014 Jun 19. PubMed PMID: 24945993.

3: Kontos AP, Elbin RJ, Lau B, Simensky S, Freund B, French J, Collins MW. Posttraumatic migraine as a predictor of recovery and cognitive impairment after sport-related concussion. Am J Sports Med. 2013 Jul;41(7):1497-504. doi: 10.1177/0363546513488751. Epub 2013 May 22. PubMed PMID: 23698389.

APPENDICES – none

SUPPORTING DATA – all figures including in body of report